Royal College of
Obstetricians and Gynaecologists

Bringing to life the best in women's health care

British Fertility Society

Scientific Impact Paper No. 8

May 2012

In Vitro Fertilisation: Perinatal Risks and Early Childhood Outcomes

In Vitro Fertilisation: Perinatal Risks and Early Childhood Outcomes

1. Introduction

It is estimated that infertility affects one in seven couples in the UK at some stage in their reproductive life.[1] After over 30 years of experience, in vitro fertilisation (IVF) is now routine medical practice in the management of infertility, and births following IVF are estimated to account for over 1% of all births in the UK. This proportion is notably lower than some European countries, reflecting the different policies of public funding for this procedure.

This paper summarises the growing body of evidence concerning the outcomes of pregnancy, delivery and early childhood associated with being conceived by IVF and related procedures. For these purposes this review will include data relating to IVF and associated procedures such as intracytoplasmic sperm injection (ICSI), blastocyst culture, assisted hatching and genetic diagnosis but will not cover gamete intrafallopian transfer (GIFT) or ovulation induction alone or with artificial insemination. Unless specifically stated, the term IVF is used to encompass IVF and any laboratory procedure associated with IVF.

2. Perinatal risks

2.1 Multiple conceptions and multiple births

The single most important determinant of pregnancy and long-term outcome is whether the pregnancy is a singleton or multiple gestation, irrespective of whether it is a natural or assisted conception. Furthermore, the risks increase disproportionately in relation to the number of fetuses, with monozygosity also being associated with higher rates of adverse outcomes. IVF itself appears to increase the risk of monozygous twins by two-fold compared with natural conception, although the incidence of monozygotic twins even after IVF remains very low.[2] At present, about one in four of all IVF pregnancies result in a multiple birth in the UK[3] owing to the common practice of replacing two or three embryos, with the vast majority of multiple pregnancies occurring after multiple embryo transfer. Multiple pregnancy is perceived as an ideal outcome by many parents and indeed for the majority of multiples, particularly twins, there are no long-term adverse consequences. However, at present it is impossible to predict who will or will not have complications perinatally or later in life. Consequently, elective single embryo transfer and limitation in the UK to the transfer of two embryos in women under the age of 40 is a population level strategy to minimise the risks of prematurity and its associated complications and socioeconomic costs. The aim of this strategy is to reduce IVF-related multiple pregnancy to less than 10% by 2012.[4] When multiple pregnancy is diagnosed in a fertility unit, referral to a specialised multiple birth clinic should be made. This would allow the couple to receive counselling about selective fetal reduction for triplets and high order pregnancies, as well as specialist prenatal screening and diagnosis.

2.2 Preterm birth

Multiple pregnancy per se is a clear risk factor for preterm birth; however, there is an additional small but statistically significant 23% increase in the relative risk of preterm birth in IVF twins compared with natural twins, although the relative contribution of spontaneous or elective preterm birth has not been identified. Similarly, although in singletons there is an estimated two-fold increased risk of preterm birth and moderate preterm birth following IVF, the contribution of spontaneous preterm labour is unknown.[5–7] Clearly, spontaneous and elective preterm births have largely different aetiologies, principally reflecting infection and placental dysfunction, respectively. Consequently, maternal and treatment factors which are associated with these will have an impact on the overall outcome. For example, duration and cause of infertility can influence the risk of preterm birth and treatment characteristics. Treatment requiring donor oocytes or ICSI can influence the risk.[8] In addition, early fetal loss in a multiple gestation can increase the risk of preterm birth for the remaining singleton.[9,10] Although

many of these factors cannot be addressed, accurate estimates of risk will improve counselling and increase the likelihood of the mother or couple choosing elective single embryo transfer.

2.3 Low birth weight and small for gestational age

Although prematurity due to multiple pregnancy is a clear risk factor for low birth weight (LBW) <2500 g, very low birth weight (VLBW) <1500 g and extremely low birth weight (ELBW) <1000 g, singleton IVF pregnancies still demonstrate an increased risk of low birth weight compared with naturally conceived singletons (RR 1.6 95%, CI 1.29–1.98; RR 2.65, 95% CI 1.83–3.84; RR 3.02, 95% CI 0.12–74.66 for each birth weight category respectively).[7] Furthermore, in IVF twin pregnancies, this increased risk is still evident compared with natural twins (LBW RR 1.14, 95% CI 1.06–1.22, VLBW RR 1.28, 95% CI 0.73–2.24 and ELBW RR 0.88, 95% CI 0.04–19.40).[11] Although prematurity will partly influence the risk of LBW in singletons, the relative risk of babies being small for gestational age is also increased by approximately 40–60%, suggesting that factors other than preterm birth are responsible.[5–7] Notably, the number of original fetal hearts observed on ultrasound is a determinant of the risk of LBW,[9,10,12] and inevitably the risk of impaired fetal growth will be partially ameliorated by elective single embryo transfer.[13] However, the characteristics of the mother and father and the treatment cycle may also influence the risk of LBW[8] and these will be harder to address.

2.4 Congenital anomalies

Between 3% and 5% of all infants are diagnosed with a congenital anomaly soon after birth. IVF is associated with a 30–40% increased risk of major congenital anomalies compared with natural conceptions.[14–16] Notably, this risk is not attributable to the increased risk of congenital anomalies associated with multiple birth, as even in singleton pregnancies the excess risk remains.[15] It appears that the increased risk is partly attributable to the underlying infertility or its determinants as couples who take longer than 12 months to conceive also exhibit an increased risk of anomalies (hazard ratio [HR] 1.20, 95% CI 1.07–1.35), although this was not as high as that observed in treated infertile couples (HR 1.39, 95% CI 1.23–1.57).[17] The principal anomalies which occur in IVF pregnancies include a range of gastrointestinal, cardiovascular and musculoskeletal defects and specifically septal heart defects, cleft lip, oesophageal atresia and anorectal atresia.[18,19] Of note, while the relative risk of major congenital anomalies associated with IVF is in the order of 30–40%, the absolute risk is nevertheless low since anomalies per se are relatively uncommon.

2.5 Vertical transmission of genetic diseases

In some cases infertility may be genetic in origin, and successful IVF treatment may therefore facilitate intergenerational transmission. This has led to concern that children born following these techniques will express a greater number of genetic abnormalities. There is an increased prevalence of structural chromosomal abnormalities in infertile men and women: a 4.6% prevalence of autosomal translocation and inversions in oligospermic men, and a 1.14% prevalence of autosomal reciprocal balanced translocations in infertile women (general population 0.16%).[20,21] However, these structural abnormalities would normally be detected and subsequent transmission avoided by use of preimplantation genetic diagnosis (PGD) following karyotyping of men with azoospermia or severe oligospermia, and in couples with recurrent implantation failure or miscarriage. Microdeletions of the long arm of the Y chromosome (Yq), in particular the AZF region, can also cause spermatogenic failure and either oligo- or azoospermia, the latter preventing further vertical transmission.[22,23] However, sons conceived from oligospermic men with Yq microdeletions will inherit this subfertile phenotype, and further expansion or de novo deletions may occur resulting in a worse phenotype in the offspring.[24–28]

Although other single gene disorders such as cystic fibrosis are associated with infertility, owing to congenital absence of the vas deferens, vertical transmission of the common mutations can be avoided through testing the female partner and performing PGD if she is a carrier. Lastly, there is increasing evidence that epigenetics may contribute to abnormal embryo and trophoblast development, with IVF

superovulation and culture conditions capable of inducing epigenetic changes and long-term genomic imprinting.[29,30] To date, nine human imprinting syndromes have been identified, but current evidence links IVF with only three: Beckwith-Wiedemann syndrome (BWS), Angelman syndrome (AS) and, more recently, maternal hypomethylation syndrome.[31] The overall incidence of these conditions is very low at less than 1 in 12 000 births (BWS 1 in 13 700, and Angelman syndrome 1 in 16 000) and consequently routine screening for imprinting disorders in children born after IVF is not recommended. Although these conditions may represent the extreme end of the epigenetic spectrum, there are potentially much subtler effects as the type of culture media used can profoundly affect birth weight in humans[32] and animals – the so-called large offspring syndrome in cattle and sheep.[33] Similarly, administration of exogenous hormones to induce oocyte maturation in vivo, as well as in vitro, may hamper the proper acquisition of maternal imprints during oogenesis and both maternal and paternal methylation-dependent imprints during embryo development in a dose-dependent manner.[34,35]

2.6 Perinatal mortality

Some caution should be taken in the interpretation of the pooled findings relating to perinatal mortality and IVF because of a particular lack of consistency in results across the studies pooled and the influence of one particularly large study.[5] However, accepting this, the overall pooled result suggests there is nearly a 70% increase in the risk of perinatal death for IVF singletons compared with natural conceptions.[5] A recent prospective follow-up study of 20 166 singleton pregnancies compared the risk of stillbirths between fertile women, subfertile women (time to natural conception greater than 12 months) and women pregnant after fertility treatment (IVF and non-IVF assisted reproductive technique [ART]).[36] Only women who conceived with IVF had a statistically significant four-fold increased risk of stillbirth compared with fertile women. This would suggest that the increased risk of stillbirth is associated with treatment-related factors to a greater degree than infertility/subfertility itself. However, this is in contrast to the findings from three studies of perinatal mortality in singletons conceived naturally following a prolonged time to conception, which found a two- to three-fold increased risk of perinatal death compared with singletons naturally conceived without a prolonged time to conception.[37–39] The results for twins are similarly inconsistent, but the combined results do suggest that, having adjusted for confounders, there is a decrease in the risk of perinatal death in IVF twins of between 16% and 42% compared with natural twins, potentially reflecting the lower incidence of monozygotic twins after IVF.[5]

3. Specific IVF and related procedures

3.1 Preimplantation genetic diagnosis/screening

Preimplantation genetic diagnosis/screening (PGD/PGS) requires the removal of one or more blastomeres from the embryo for genetic testing, thereby allowing the transfer of unaffected embryos. Despite the removal of a variable amount of material from the developing embryo, the overall prospect for children appears in line with standard ICSI.[40,41] However, PGD/PGS has been associated with a substantial increased risk of perinatal death compared with standard ICSI (4.6% versus 1.9%; OR 2.56). When stratified for multiple births, perinatal death rates among PGD/PGS singleton and ICSI singleton children were similar (1% versus 1.3%), but there were significantly more perinatal deaths seen in post-PGD/PGS multiple pregnancies compared with conventional ICSI multiple pregnancies (11.7% versus 2.5%), and this was not attributable to an increased prevalence of monozygotic twins.[42] Notably, all of these perinatal mortality rates are substantially higher than the general population rates, and further research is required to elucidate the time of death to determine whether timing of induction of labour could be altered to minimise the risk at term.

3.2 Blastocyst culture

The extended culture of embryos in vitro from the traditional day 2 (cleavage-stage) transfer date to day 5 (blastocyst stage) has been advocated as a means of improving embryo selection and thus pregnancy rates. The embryo is sequentially cultured in different media to facilitate in vitro development. In a

Cochrane review, the evidence of a significant difference in live-birth rate per couple between the two treatment groups was detected in favour of blastocyst culture (day 2/3: 29.4% versus day 5/6: 36.0%).[43] A systematic review and meta-analysis has demonstrated that the probability of live birth after fresh IVF is 40% higher after blastocyst-stage embryo transfer compared with cleavage-stage embryo transfer when equal number of embryos are transferred.[44]

Studies in different animal models have raised some concerns about potential deleterious effects of extended embryo culture, including imprinting disorders, overgrowth, behavioural abnormalities as well as cardiovascular and metabolic dysfunction.[45–48] In the human, a sex ratio in favour of males has been associated with blastocyst culture.[49] After adjusting for confounders, the risk of preterm birth among singletons was significantly greater after blastocyst-stage transfer than after cleavage-stage transfer; the risk of congenital malformations was also significantly higher.[50] The odds of any congenital malformation versus population figures was 50% higher after blastocyst transfer whereas after cleavage-stage transfer it was only 11% higher. These differences in outcome may be attributable to an increase in monozygotic twinning; however, there are conflicting data regarding blastocyst transfer and the risk of monozygotic twinning. A retrospective study has demonstrated that monozygotic twinning is not increased after single blastocyst transfer compared with single cleavage-stage embryo transfer,[51] whereas other authors have concluded that blastocyst culture is associated with an increased risk of monozygotic twinning.[49,52] These differences might be related to a variation in culture media. Elective single blastocyst transfer is an effective option to decrease the risk of multiple birth while maintaining a high pregnancy rate. Nevertheless, the possible risks associated with the extended period in culture require continuing surveillance of the children born after blastocyst transfer.

3.3 Assisted hatching

Assisted hatching (AH), a technique whereby the zona pellucida is disrupted to facilitate implantation, has recently been shown to enhance clinical pregnancy rates (OR 1.29, 95% CI 1.12–1.49) but not live birth (OR 1.13, 95% CI 0.83–1.55) nor to reduce multiple pregnancy rates (OR 1.67, 95% CI 1.24–2.26).[53,54] The long-term impact on the pregnancy and offspring is, however, unknown.

3.4 In vitro maturation

Immature oocyte retrieval and subsequent oocyte maturation in vitro (IVM) without any ovarian stimulation is a new development in ART. IVM gives the benefits of ovarian stimulation – namely, more oocytes – without the risks of ovarian stimulation. Patients with polycystic ovary syndrome (PCOS) or polycystic ovaries represent a good indication for IVM. Over 1000 babies have been born worldwide following IVM and, although formal prospective paediatric follow-up studies are limited, similar obstetric outcomes and congenital anomaly rates between babies born following IVM, IVF and ICSI have been reported.[55] Neurological development of IVM children at 2 years of age also appears to be normal, although this conclusion is based on only 67 children.[56,57]

4. Long-term outcomes for children

The physical, neurological and developmental health of children born after IVF is certainly one of the most important aspects when discussing the potential adverse effects of IVF.[58] Overall, the neuromotor, cognitive, language and behavioural outcomes in children born following IVF or ICSI appears to be similar to children conceived naturally.[59–62] The only consistent adverse finding has been an increased risk of cerebral palsy, which is partly, but not wholly, explained by the increased risk of preterm delivery (unadjusted OR 2.18, 95% CI 1.71–2.77).[63] With respect to children's growth, there appears to be little impact at age 12.[64]

The Barker hypothesis predicts that adverse antenatal conditions can lead to long-term consequences in the adult. For example, under-nutrition during pregnancy is associated with an increased risk of coronary heart disease, type 2 diabetes, stroke and hypertension.[65] It is therefore legitimate to question if IVF might

be associated with cardiometabolic disturbances occurring in adulthood or even earlier during adolescence. Ceelen et al. have observed that peripheral adipose tissue mass was higher in IVF children.[66] The same authors have also reported cardiometabolic differences in children born after IVF, with approximately a 4 mmHg and 2 mmHg increase in systolic and diastolic blood pressure respectively and an increase in fasting glucose, with none of these differences explained by confounders including current body size, birth weight, and other early life factors or by parental characteristics, including subfertility cause.[67] Moreover, these authors have suggested that early childhood growth (weight gain) might predict cardiovascular risk factors (blood pressure and body fat composition) in IVF children.[68] These important data strongly emphasise the need for metabolic epidemiological studies in IVF adolescents and adults.

5. Maternal morbidity

5.1 Maternal age

Increasing maternal age is a risk factor for almost all pregnancy and perinatal complications. The average age at which women attempt to conceive continues to rise and consequently IVF is increasingly used by older women who are already predisposed to pregnancy complications. However, even when comparing age-matched controls there appears to be an increased risk of complications associated with infertility, with a higher rate of caesarean section delivery, obstetric haemorrhage, pre-eclampsia, pregnancy-induced hypertension and gestational diabetes all noted in older women having IVF.[69,70] All of these conditions are associated with unfavourable perinatal outcomes for the neonate, including preterm delivery, low birth weight and admission for neonatal intensive care. By virtue of their age, older women are more likely to have pre-existing comorbidities further complicating their pregnancy course and outcome. Importantly, women with significant comorbidities, regardless of their age, should receive pre-IVF assessment and counselling.

5.2 Recipients of donor oocytes

With delays in the age at which women attempt to conceive, an increasing number of women are using oocyte donation, a technique previously limited to women with premature ovarian failure. Again there appears to be an increase in early pregnancy and perinatal complications, with the risk of pregnancy-induced hypertension in particular ranging from 16% to 40% of cases, with the greatest risk observed in primiparous women.[12,71] Despite these risks, there is almost no information on the long-term outcomes of egg donation pregnancies for the mother and her child.

5.3 Polycystic ovary syndrome

PCOS, as defined by the 2003 Rotterdam criteria, is a common condition affecting 6–10% of reproductive-aged women.[72–74] Metabolic syndrome is often encountered in PCOS and obesity is a classic clinical manifestation. Long-term risks include an increased risk of type 2 diabetes and there is some evidence of an increased risk of cardiovascular events.[75,76] In these women, the presence of infertility caused by anovulation often requires treatment such as ovulation induction with clomifene citrate, ovarian stimulation with gonadotrophin and IVF. Pregnancy outcomes in women with PCOS were analysed in a meta-analysis.[77] The results indicate an increased risk of gestational diabetes (OR 2.94, 95% CI 1.70–5.08), pregnancy-induced hypertension (OR 3.67, 95% CI 1.98–6.81), pre-eclampsia (OR 3.47, 95% CI 1.95–6.17) and preterm birth (OR 1.75, 95% CI 1.16–2.62). In addition, an increased risk of admission to neonatal intensive care (OR 2.31, 95% CI: 1.25–4.26) and higher perinatal mortality (OR 3.07, 95% CI 1.03–9.21), which were not related to multiple births, have been observed. These data highlight the importance of pre-ART counselling of women with PCOS and emphasise the need for weight management before ART.

6. Antenatal screening

Use of first-trimester combined ultrasound and biochemical screening for Down syndrome is now recommended;[78] however, the effect of IVF is largely ignored. Notably, pregnancy-associated plasma

protein-A (PAPP-A) levels are significantly lower in fresh transfer IVF pregnancies, the consequence of which is an increased risk of receiving a false-positive result and increased odds of having a chorionic villous sampling or amniocentesis.[79,80] However, this increase in false-positive rate was not confirmed in a recent, smaller, study.[81] This may reflect the relative inaccuracy of ultrasound dating, with the precise dating available for IVF pregnancies being largely ignored.[82–84] Further larger studies will be required before recommendations for adjustment of risk calculations for IVF pregnancies can be made.

7. Contribution of the subfertile phenotype

Although many of the above studies have used normal controls as the comparator, it is increasingly clear that the factors which predispose to infertility are also linked with adverse perinatal outcomes, with subfertility acting as a proxy for this. Therefore, in the future researchers trying to determine the individual effect of a novel technique or infertility treatment should consider also using women with a prolonged time to natural conception as the comparison group.[60,85] This will allow delineation of whether the IVF procedure itself or factors inherent to the couple and their infertility are associated with any adverse outcome.

8. Opinion

While it is clear that IVF pregnancies are at increased risk of adverse perinatal outcomes, it is also the case that the majority of the children born following IVF will have a good outcome. For those with poorer outcomes this inevitably reflects aspects of the treatment but also the interplay with the underlying features that the couple bring to the pregnancy. Further research is needed to untangle this complex relationship to allow effective targeted interventions. Given the known risks associated with IVF pregnancies, risk assessment is required during antenatal care with appropriate referral.[86] The adoption of elective single embryo transfer provides a clear example of how treatment strategies can be altered to improve outcomes. At present the long-term follow-up studies on children born following IVF are largely reassuring once the confounding factors of prematurity and multiple gestation are removed. However, with the continued refinement of the technical process and clinical application of novel developments, continued surveillance is a prerequisite.

References

1. Bhattacharya S, Porter M, Amalraj E, Templeton A, Hamilton M, Lee AJ, Kurinczuk JJ. The epidemiology of infertility in the North East of Scotland. *Hum Reprod* 2009;24:3096–107.
2. Vitthala S, Gelbaya TA, Brison DR, Fitzgerald CT, Nardo LG. The risk of monozygotic twins after assisted reproductive technology: a systematic review and meta-analysis. *Human Reprod Update* 2009;15:45–55.
3. Human Fertilisation & Embryology Authority. Latest UK IVF figures – 2008. London: HFEA; 2011.
4. Human Fertilisation & Embryology Authority. *Multiple births and single embryo transfer review*. London: HFEA; 2011 [http://www.hfea.gov.uk/530.html]
5. Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ* 2004;328:261.
6. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol* 2004;103:551–63.
7. McDonald SD, Han Z, Mulla S, Murphy KE, Beyene J, Ohlsson A. Preterm birth and low birth weight among in vitro fertilization singletons: a systematic review and meta-analyses. *Eur J Obstet Gynecol Reprod Biol* 2009;146:138–48.
8. Nelson SM, Lawlor DA. Predicting live birth, preterm delivery, and low birth weight in infants born from in vitro fertilisation: a prospective study of 144,018 treatment cycles. *PLoS Med* 2011;8:e1000386.

9. Dickey RP, Taylor SN, Lu PY, Sartor BM, Storment JM, Rye PH, et al. Spontaneous reduction of multiple pregnancy: incidence and effect on outcome. *Am J Obstet Gynecol* 2002;186:77–83.
10. Pinborg A, Lidegaard O, la Cour Freiesleben N, Andersen AN. Consequences of vanishing twins in IVF/ICSI pregnancies. *Hum Reprod* 2005;20:2821–9.
11. McDonald SD, Han Z, Mulla S, Ohlsson A, Beyene J, Murphy KE. Preterm birth and low birth weight among in vitro fertilization twins: a systematic review and meta-analyses. *Eur J Obstet Gynecol Reprod Biol* 2010;148:105–13.
12. Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med* 2002;346:731–7.
13. De Neubourg D, Gerris J, Mangelschots K, Van Royen E, Vercruyssen M, Steylemans A, et al. The obstetrical and neonatal outcome of babies born after single-embryo transfer in IVF/ICSI compares favourably to spontaneously conceived babies. *Hum Reprod* 2006;21:1041–6.
14. Rimm AA, Katayama AC, Diaz M, Katayama KP. A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. *J Assist Reprod Genet* 2004;21:437–43.
15. Hansen M, Bower C, Milne E, de Klerk N, Kurinczuk JJ. Assisted reproductive technologies and the risk of birth defects – a systematic review. *Hum Reprod* 2005;20:328–38.
16. McDonald S, Murphy K, Beyene J, Ohlsson A. Perinatal outcomes of in vitro fertilization twins: a systematic review and meta-analyses. *Am J Obstet Gynecol* 2005;193:141–52.
17. Zhu JL, Basso O, Obel C, Bille C, Olsen J. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. *BMJ* 2006;333:679.
18. Reefhuis J, Honein MA, Schieve LA, Correa A, Hobbs CA, Rasmussen SA. Assisted reproductive technology and major structural birth defects in the United States. *Hum Reprod* 2009;24:360–6.
19. El-Chaar D, Yang Q, Gao J, Bottomley J, Leader A, Wen SW, et al. Risk of birth defects increased in pregnancies conceived by assisted human reproduction. *Fertil Steril* 2009;92:1557–61.
20. Schreurs A, Legius E, Meuleman C, Fryns JP, D'Hooghe TM. Increased frequency of chromosomal abnormalities in female partners of couples undergoing in vitro fertilization or intracytoplasmic sperm injection. *Fertil Steril* 2000;74:94–6.
21. Clementini E, Palka C, Iezzi I, Stuppia L, Guanciali-Franchi P, Tiboni GM. Prevalence of chromosomal abnormalities in 2078 infertile couples referred for assisted reproductive techniques. *Hum Reprod* 2005;20:437–42.
22. Krausz C, Degl'Innocenti S. Y chromosome and male infertility: update, 2006. *Front Biosci* 2006;11:3049–61.
23. Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. *Int J Androl* 2004;27:240–9.
24. Silber SJ, Alagappan R, Brown LG, Page DC. Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. *Hum Reprod* 1998;13:3332–7.
25. Kamischke A, Gromoll J, Simoni M, Behre HM, Nieschlag E. Transmission of a Y chromosomal deletion involving the deleted in azoospermia (DAZ) and chromodomain (CDY1) genes from father to son through intracytoplasmic sperm injection: case report. *Hum Reprod* 1999;14:2320–2.
26. Page DC, Silber S, Brown LG. Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. *Hum Reprod* 1999;14:1722–6.
27. Cram DS, Ma K, Bhasin S, Arias J, Pandjaitan M, Chu B, et al. Y chromosome analysis of infertile men and their sons conceived through intracytoplasmic sperm injection: vertical transmission of deletions and rarity of de novo deletions. *Fertil Steril* 2000;74:909–15.

28. Lee SH, Ahn SY, Lee KW, Kwack K, Jun HS, Cha KY. Intracytoplasmic sperm injection may lead to vertical transmission, expansion, and de novo occurrence of Y-chromosome microdeletions in male fetuses. *Fertil Steril* 2006;85:1512–5.
29. Laprise SL. Implications of epigenetics and genomic imprinting in assisted reproductive technologies. *Mol Reprod Dev* 2009;76:1006–18.
30. Wilkins-Haug L. Epigenetics and assisted reproduction. *Curr Opin Obstet Gynecol* 2009;21:201–6.
31. Amor DJ, Halliday J. A review of known imprinting syndromes and their association with assisted reproduction technologies. *Hum Reprod* 2008;23:2826–34.
32. Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, et al. Effect of in vitro culture of human embryos on birthweight of newborns. *Hum Reprod* 2010;25:605–12.
33. Young LE, Sinclair KD, Wilmut I. Large offspring syndrome in cattle and sheep. *Rev Reprod* 1998;3:155–63.
34. Sato A, Otsu E, Negishi H, Utsunomiya T, Arima T. Aberrant DNA methylation of imprinted loci in superovulated oocytes. *Hum Reprod* 2007;22:26–35.
35. Market-Velker BA, Zhang L, Magri LS, Bonvissuto AC, Mann MR. Dual effects of superovulation: loss of maternal and paternal imprinted methylation in a dose-dependent manner. *Hum Mol Gen* 2010;19:36–51.
36. Wisborg K, Ingerslev HJ, Henriksen TB. IVF and stillbirth: a prospective follow-up study. *Hum Reprod* 2010;25:1312–6.
37. Jaques AM, Amor DJ, Baker HW, Healy DL, Ukoumunne OC, Breheny S, et al. Adverse obstetric and perinatal outcomes in subfertile women conceiving without assisted reproductive technologies. *Fertil Steril* 2010;94:2674–9.
38. Draper ES, Kurinczuk JJ, Abrams KR, Clarke M. Assessment of separate contributions to perinatal mortality of infertility history and treatment: a case-control analysis. *Lancet* 1999;353:1746–9.
39. Basso O, Olsen J. Subfecundity and neonatal mortality: longitudinal study within the Danish national birth cohort. *BMJ* 2005;330:393–4.
40. Liebaers I, Desmyttere S, Verpoest W, De Rycke M, Staessen C, Sermon K, et al. Report on a consecutive series of 581 children born after blastomere biopsy for preimplantation genetic diagnosis. *Hum Reprod* 2010;25:275–82.
41. Harper JC, Coonen E, De Rycke M, Harton G, Moutou C, Pehlivan T, et al. ESHRE PGD Consortium data collection X: cycles from January to December 2007 with pregnancy follow-up to October 2008. *Hum Reprod* 2010;25:2685–707.
42. Verpoest W, Van Landuyt L, Desmyttere S, Cremers A, Devroey P, Liebaers I. The incidence of monozygotic twinning following PGD is not increased. *Hum Reprod* 2009;24:2945–50.
43. Blake DA, Farquhar CM, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. *Cochrane Database Syst Rev* 2007;(4):CD002118.
44. Papanikolaou EG, Kolibianakis EM, Tournaye H, Venetis CA, Fatemi H, Tarlatzis B, et al. Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis. *Hum Reprod* 2008;23:91–9.
45. Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, et al. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. *Nat Genet* 2001;27:153–4.
46. Fernandez-Gonzalez R, Moreira P, Bilbao A, Jimenez A, Perez-Crespo M, Ramirez MA, et al. Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. *Proc Natl Acad Sci U S A* 2004;101:5880–5.
47. Ecker DJ, Stein P, Xu Z, Williams CJ, Kopf GS, Bilker WB, et al. Long-term effects of culture of preimplantation mouse embryos on behavior. *Proc Natl Acad Sci U S A* 2004;101:1595–600.
48. Watkins AJ, Platt D, Papenbrock T, Wilkins A, Eckert JJ, Kwong WY, et al. Mouse embryo culture induces changes in postnatal phenotype including raised systolic blood pressure. *Proc Natl Acad Sci U S A* 2007;104:5449–54.

49. Chang HJ, Lee JR, Jee BC, Suh CS, Kim SH. Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: a systematic review and meta-analysis. *Fertil Steril* 2009;91:2381–90.
50. Källén B, Finnström O, Lindam A, Nilsson E, Nygren KG, Olausson PO. Blastocyst versus cleavage stage transfer in in vitro fertilization: differences in neonatal outcome? *Fertil Steril* 2010;94:1680–3.
51. Papanikolaou EG, Fatemi H, Venetis C, Donoso P, Kolibianakis E, Tournaye H, et al. Monozygotic twinning is not increased after single blastocyst transfer compared with single cleavage-stage embryo transfer. *Fertil Steril* 2010;93:592–7.
52 Kawachiya S, Bodri D, Shimada N, Kato K, Takehara Y, Kato O. Blastocyst culture is associated with an elevated incidence of monozygotic twinning after single embryo transfer. *Fertil Steril* 2011;95:2140–2.
53. Das S, Blake D, Farquhar C, Seif MM. Assisted hatching on assisted conception (IVF and ICSI). *Cochrane Database Syst Rev* 2009; (2):CD001894.
54. Martins WP, Rocha IA, Ferriani RA, Nastri CO. Assisted hatching of human embryos: a systematic review and meta-analysis of randomized controlled trials. *Hum Reprod Update* 2011;17:438–53.
55. Buckett WM, Chian RC, Holzer H, Dean N, Usher R, Tan SL. Obstetric outcomes and congenital abnormalities after in vitro maturation, in vitro fertilization, and intracytoplasmic sperm injection. *Obstet Gynecol* 2007;110:885–91.
56. Shu-Chi M, Jiann-Loung H, Yu-Hung L, Tseng-Chen S, Ming IL, Tsu-Fuh Y. Growth and development of children conceived by in-vitro maturation of human oocytes. *Early Hum Dev* 2006;82:677–82.
57. Söderström-Anttila V, Salokorpi T, Pihlaja M, Serenius-Sirve S, Suikkari AM. Obstetric and perinatal outcome and preliminary results of development of children born after in vitro maturation of oocytes. *Hum Reprod* 2006;21:1508–13.
58. Hvidtjørn D, Grove J, Schendel DE, Vaeth M, Ernst E, Nielsen LF, et al. Cerebral palsy among children born after in vitro fertilization: the role of preterm delivery – a population-based, cohort study. *Pediatrics* 2006;118:475–82.
59. Carson C, Kurinczuk JJ, Sacker A, Kelly Y, Klemetti R, Redshaw M, et al. Cognitive development following ART: effect of choice of comparison group, confounding and mediating factors. *Hum Reprod* 2010;25:244–52.
60. Middelburg KJ, Heineman MJ, Bos AF, Hadders-Algra M. Neuromotor, cognitive, language and behavioural outcome in children born following IVF or ICSI – a systematic review. *Hum Reprod Update* 2008;14:219–31.
61. Belva F, Henriet S, Liebaers I, Van Steirteghem A, Celestin-Westreich S, Bonduelle M. Medical outcome of 8-year-old singleton ICSI children (born >or=32 weeks' gestation) and a spontaneously conceived comparison group. *Hum Reprod* 2007;22:506–15.
62. Leunens L, Celestin-Westreich S, Bonduelle M, Liebaers I, Ponjaert-Kristoffersen I. Follow-up of cognitive and motor development of 10-year-old singleton children born after ICSI compared with spontaneously conceived children. *Hum Reprod* 2008;23:105–11.
63. Hvidtjørn D, Schieve L, Schendel D, Jacobsson B, Svaerke C, Thorsen P. Cerebral palsy, autism spectrum disorders, and developmental delay in children born after assisted conception: a systematic review and meta-analysis. *Arch Pediatr Adolesc Med* 2009; 163:72–83.
64. Basatemur E, Shevlin M, Sutcliffe A. Growth of children conceived by IVF and ICSI up to 12 years of age. *Reprod Biomed Online* 2010;20:144–9.
65. Barker DJ. The developmental origins of adult disease. *J Am Coll Nutr* 2004;23:588S–595S.
66. Ceelen M, van Weissenbruch MM, Roos JC, Vermeiden JP, van Leeuwen FE, Delemarre-van de Waal HA. Body composition in children and adolescents born after in vitro fertilization or spontaneous conception. *J Clin Endocrinol Metab* 2007;92:3417–23.

67. Ceelen M, van Weissenbruch MM, Vermeiden JP, van Leeuwen FE, Delemarre-van de Waal HA. Cardiometabolic differences in children born after in vitro fertilization: follow-up study. *J Clin Endocrinol Metab* 2008;93:1682–8.
68. Ceelen M, van Weissenbruch MM, Prein J, Smit JJ, Vermeiden JP, Spreeuwenberg M, et al. Growth during infancy and early childhood in relation to blood pressure and body fat measures at age 8-18 years of IVF children and spontaneously conceived controls born to subfertile parents. *Hum Reprod* 2009;24:2788–95.
69. Reddy UM, Wapner RJ, Rebar RW, Tasca RJ. Infertility, assisted reproductive technology, and adverse pregnancy outcomes: executive summary of a National Institute of Child Health and Human Development workshop. *Obstet Gynecol* 2007;109:967–77.
70. Healy DL, Breheny S, Halliday J, Jaques A, Rushford D, Garrett C, et al. Prevalence and risk factors for obstetric haemorrhage in 6730 singleton births after assisted reproductive technology in Victoria Australia. *Hum Reprod* 2010;25:265–74.
71. Wiggins DA, Main E. Outcomes of pregnancies achieved by donor egg in vitro fertilization – a comparison with standard in vitro fertilization pregnancies. *Am J Obstet Gynecol* 2005;192:2002–2006; discussion 2006–2008.
72. The Rotterdam ESHRE/ASRM sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004;19:41–7.
73. Asunción M, Calvo RM, San Millán JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *J Clin Endocrinol Metab* 2000;85:2434–8.
74. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab* 2004;89:2745–9.
75. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab* 1999;84:165–9.
76. Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. *J Clin Endocrinol Metab* 2010;95:2038–49.
77. Boomsma CM, Eijkemans MJ, Hughes EG, Visser GH, Fauser BC, Macklon NS. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. *Hum Reprod Update* 2006;12:673–83.
78. Screening for Downs syndrome: UK NSC Policy recommendations 2011–2014 Model of Best Practice [http://fetalanomaly.screening.nhs.uk/standardsandpolicies].
79. Amor DJ, Xu JX, Halliday JL, Francis I, Healy DL, Breheny S, et al. Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome. *Hum Reprod* 2009;24:1330–8.
80. Gjerris AC, Loft A, Pinborg A, Christiansen M, Tabor A. First-trimester screening markers are altered in pregnancies conceived after IVF/ICSI. *Ultrasound Obstet Gynecol* 2009;33:8–17.
81. Matilainen M, Peuhkurinen S, Laitinen P, Jarvela I, Morin-Papunen L, Ryynanen M. In combined first-trimester Down syndrome screening, the false-positive rate is not higher in pregnancies conceived after assisted reproduction compared with spontaneous pregnancies. *Fertil Steril* 2011;95:378–81.
82. Wennerholm UB, Bergh C, Hagberg H, Sultan B, Wennergren M. Gestational age in pregnancies after in vitro fertilization: comparison between ultrasound measurement and actual age. *Ultrasound Obstet Gynecol* 1998;12:170–4.

83. Tunón K, Eik-Nes SH, Grøttum P, Von Düring V, Kahn JA. Gestational age in pregnancies conceived after in vitro fertilization: a comparison between age assessed from oocyte retrieval, crown-rump length and biparietal diameter. *Ultrasound Obstet Gynecol* 2000;15:41–6.
84. Gjerris AC, Loft A, Pinborg A, Tabor A, Christiansen M. 2008 First-trimester screening in pregnancies conceived by assisted reproductive technology: significance of gestational dating by oocyte retrieval or sonographic measurement of crown-rump length. *Ultrasound Obstet Gynecol* 2008;32:612–7.
85. Kurinczuk JJ, Hansen M, Bower C. Methodological considerations when designing studies to examine the health of children born following ART. In: Sutcliffe AG (Editor), *Health and Welfare of ART Children*. Oxford: Informa UK Ltd, 2006. p. 11–41.
86. Pathways for Maternity Care, NHS, Quality Improvement Scotland, 2009 [http://www.healthcareimprovementscotland.org/programmes/reproductive,_maternal__child/programme_resources/keeping_childbirth_natural.aspx].

This opinion paper was produced on behalf of the Royal College of Obstetricians and Gynaecologists by:
Dr NR Vulliemoz, Institute of Reproductive Sciences, Oxford, Mr E McVeigh FRCOG, Oxford and Dr J Kurinczuk MD MSc FFPH, National Perinatal Epidemiology Unit, Oxford

and peer-reviewed by:
Mr PRS Brinsden FRCOG, President, British Fertility Society; Professor T Fleming, Professor of Developmental Biology Southampton; Professor AH Balen FRCOG, Leeds; Professor MD Kilby FRCOG, President, BMFMS.

The Scientific Advisory Committee lead reviewer was:
Professor SM Nelson MRCOG, Glasgow.

The final version is the responsibility of the Scientific Advisory Committee of the RCOG.

The review process will commence in 2015 unless otherwise indicated.

